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A STUDY OF FUNGI FOUND IN BUTTER.

BY M. GRIMES,
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V. C. E. KENNELLY AND H. A. CUMMINS,
Department of Botany,
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Price Two Shillings.

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(Plates 22 and 23.)

[Read June 26. Published separately October 31, 1930.]

In the course of an investigation into the causes of deterioration of butter made from sweet cream, eight half-pound samples of butter were forwarded by the Butter-Testing Station, Department of Agriculture, Irish Free State, for examination each month, commencing October, 1929. These eight samples were taken from the four boxes scoring highest for flavour and the four boxes scoring lowest for flavour at the first surprise inspection held each month. The percentage of salt in the butter samples varied from nil to 2·0 per cent., with the exception of a few samples containing from 2·0 per cent. to 3·0 per cent. salt.

The samples of butter for these butter inspections are taken as follows:— Inspectors pay surprise visits to creameries, take and seal a 56 lb. box of butter at each, and leave instructions to have them forwarded to the Butter-Testing Station, Dublin. These boxes are held for a period of twelve days, and then judged and scored.

The samples of butter, on the day of arrival at University College, Cork, were cut with a sterile knife, about 30 grams were taken from the interior, put into a sterile bottle, melted at 37° C., and 0·1 c.c. and 0·01 c.c., respectively, of the mixed sample plated in duplicate on nutrient lactose agar, to which sufficient lactic acid had been added to give the media a *pH* of 3·5 in order to prevent bacterial growth. The Petri dishes were then incubated for a week at 21° C. before examination. At each examination a survey was made of the different types of moulds found, and a study made of each type on its first appearance. The most prevalent species found, in order of frequence, were Penicillium, *Oidium lactis*, Aspergillus, Cladosporium, Phoma. The other species were rarely found, and there were usually only 10 or 20 present per c.c. of butter examined.

Dr. G. Van B. Gilmour, Dairy Bacteriologist, Butter-Testing Station, Department of Agriculture, Dublin, also kindly forwarded cultures of moulds isolated by him from butter for identification.

The moulds for cultivation purposes were grown on nutrient lactose agar *pH* 7·0, or on Czapek agar *pH* 6·9, or on Richards' agar (1). The Czapek solution, without the addition of agar, was used for the cultivation of the moulds in liquid, and for the study of heat tolerance and salt tolerance. The Czapek solution without the addition of agar had a *pH* of 7·5, but, as all the moulds studied grew well in it, the *pH* was not corrected to 7·0. All the *pH* figures were obtained colorimetrically by the use of a comparator, using a suitable indicator, e.g., Brom-thymol blue was used for the range 6·0 to 7·6.

The study of mould growth at low temperatures was carried out by inoculating in duplicate on nutrient lactose agar slopes, incubating at 21° C. until growth was well started, marking area of growth, and incubating one set of nutrient lactose agar slopes at 5° C. or 8° C. for three weeks in a humid atmosphere, then examining, noting if area of growth had increased, and comparing with the second set of slopes which had been held at 21° C. as controls.

Heat tolerance was studied by inoculating into Czapek solution contained in 6″ × 1″ test tubes, one-third full, heating to the desired temperature, incubating at 21° C. for two weeks, and noting if growth occurred. The *pH* of the solution was noted after period of incubation.

Salt tolerance was studied by adding 3 per cent., 5 per cent., 10 per cent., 15 per cent., and 20 per cent. of sodium chloride to Czapek solution, inoculating with a culture of the mould, incubating at 21° C. for two weeks, observing if growth occurred, and noting the *pH* before and after period of incubation.

Liquefaction of gelatine was determined by making a stab inoculation into nutrient gelatine, incubating at 21° C., and examining daily for ten days. The production of diastase by the mould species was determined by making a streak inoculation on lactose nutrient agar containing 0·1 per cent. soluble starch, incubating at 21° C., until there was marked growth, then flooding the plate with Lugol's iodine solution, and determining the size of the area that did not stain on the addition of the iodine solution. Diastase production is classed as either absent, feeble, fair, or good.

Growth in milk was determined by inoculation into litmus and calcified milk, and incubating at 21° C. for a period up to two weeks.

Where the morphology of a mould species is not described a reference is given to its description in our previous paper (2).

It is noted that the growth of moulds is affected by various factors. Tomkins (3) has shown that the rate of germination varies for different fungi, and that the further removed the temperature is from the optimum the slower the rate of germination; also, that the range of humidities over which germination is possible varies with the temperature, and that the further the tem-

perature is removed from the optimum the narrower is the range of humidities allowing germination.

Brooks and Hansford (4) observed that the spores of all mould fungi found on cold-store meat, notably *Cladosporium herbarum* (*Hormodendron cladosporioides*), *Penicillium expansum*, and *Thamnidium* spp., remain alive after being subjected to a temperature of – 6° C. for two years, and that *Mucor* spp. will grow at 0° C., or just above.

Joslyn and Cruess (5), studying various species of Penicillium, found that the salt tolerance was related to the *pH* of the medium. The lower the *pH* the less the amount of salt necessary to inhibit growth. They give the following figures for the percentage of salt required to inhibit growth with varying *pH* :—

pH	2·5	3·2	5·1	6·3
Percentage of salt ...	0	10	18	16

They also found that the thermal death-point was influenced by the *pH*, salt concentration, and the amount of inoculum used. They found that the death temperature of a penicillium species was lowered to 55° C. from 75° C. by the addition of 10 per cent. salt, and was lowered to 50° C. from 75° C. by altering the *pH* to 2·75.

One of us (6) has studied the influence of *Oidium lactis* on the keeping quality of butter made from the ripened cream containing 1·5 to 2·1 per cent. NaCl, and stored at – 6° F. for from six to seven months. He found that *Oidium lactis* did not survive cold storage.

That moulds are influenced by the percentage of NaCl in butter is also shown by Macy (7), who found that, when mould estimations were made of butter before and after cold storage for periods of one to nine months, the moulds decreased to a greater extent in the salted butter than in the unsalted. He gives the following table :—

	Mould Count.		
	Per cent. increase.	Per cent. no change.	Per cent. decrease.
Salted butter ...	22·6	12·6	64·8
Unsalted butter ...	85·4	4·0	10·6

Macy notes that the addition of 1 per cent. NaCl to the butter had an inhibitive effect. In judging the salt tolerance of moulds it must be remembered that, assuming the average amount of water in butter to be 15·5 per cent., this would be equal to 6·45 per cent. NaCl in the serum. Orla Jensen (8) states that *Oidium lactis, Penicillium glaucum,* and *Cladosporium butyri* grow better and more quickly in unsalted than in salted butter. Thom and Shaw (9) state that species of Oidium, Alternaria, and Cladosporium cannot develop in butter containing 2·5 per cent. NaCl. The following fungi are stated by Clinton (10)

to have been found in mouldy unsalted butter:—Mucor, Alternaria, *Penicillium roqueforti, Oidium lactis,* Epicoccum; and Morgan (11), who has made a study of moulds in unsalted butter, lists the following species:—*Penicillium glaucum,* Stemphylium, *Fusarium lactis, Oidium lactis, Cladosporium herbarum, Cladosporium butyri.* He states that the spores of *Oidium lactis* will remain virile for about six weeks after manufacture, and that after six weeks the mould gradually dies out. Morgan states that germination of Stemphylium spores had never occurred at a temperature higher than 70 °C., and that moulds of the Stemphylium type are capable of germinating, and growing well in media containing 5 to 7 per cent. of NaCl; also that spores of Stemphylium mould inoculated into butter showed no signs of growth when the butter was kept at 3° C. to 5° C. for three months. Morgan found that spores of *Fusarium lactis* did not germinate from cultures heated higher than 60° C., and that they are capable of germination in media containing 8 per cent. NaCl. He found that spores of *Penicillium glaucum* frequently germinated after heating to 75° C., and that the spores could germinate in media containing 7 per cent. NaCl, but rarely in media containing 8 per cent. NaCl. Morgan found that spores of *Oidium lactis* rarely germinated from cultures heated to 60° C. or over, and that no germination was found in media containing more than 4 per cent. NaCl. He states that spores of *Oidium lactis* will not germinate when kept at 5° C. for three months, but that the mould will germinate in a week at 10° C. Morgan states that spores of *Cladosporium* spp. will not germinate if heated to 70° C. or over, and will germinate in a medium containing 7 per cent. NaCl. Macy (17) made a study, using ten species of mould isolated from butter, of some of the factors influencing the growth of mould in butter, and concluded that, as an ample food and water supply is provided, the growth of moulds in butter appears to depend largely upon the species of mould, the humidity of the atmosphere, the supply of oxygen, the temperature of storage, time, and the concentration of salt.

These influences may act separately or collectively.

Aspergillus fumigatus Fres. (1, p. 129) (Pl. 22, figs. 2 and 4, Pl. 23, fig. 1).

Colonies on Czapek agar velvety or floccose, green to dark green, or black when old. Reverse of colony uncoloured or rose red on lactose agar slopes, and also in Czapek solution when heated to 70° C.

Conidiophores short, crowded, greenish coloured, rising from submerged or aerial hyphae, septate or unseptate. The vesicle is flask-shaped, 10μ in diameter (Thom (1, p. 129) states 20μ to 30μ is usual size), fertile on upper half. The sterigmata are in one series, varying in size, and form solid columns. The condidia are dark green, globose, 2–3·5μ in diameter. Cultures grow well at 37° C. The forms and measurements of *A. fumigatus* are very variable; the heads, however, have a characteristic clavate shape, and are much larger than the somewhat similar heads formed in old cultures of *Aspergillus terreus.*

Milk Cultures.—Good growth, coagulation, fine curd. No odour.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 80° C. No apparent growth of colony when kept three weeks under 5° C. No red coloration in medium while in cold storage: this, however, appeared when the culture was transferred to a cupboard at room temperature (18° C.), when there was normal growth.

Salt Tolerance.—Growth in Czapek solution containing 10 per cent. NaCl fairly strong; practically no growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—Little or no change when grown for two weeks in Czapek solution with or without the addition of salt.

Gelatine.—Slight liquefaction in 10 days.

Diastase Production.—Fair.

Aspergillus luteo-niger (Lutz) (1, p. 166) (2, p. 315).

Milk Cultures.—Acid, coagulation followed by rapid digestion.

Heat Tolerance.—Growth when heated to 75° C. No growth when heated to 80° C. Slight growth when kept at about 8° C. for three weeks. When kept at 5° C. for three weeks there was no appreciable increase in size of colony.

Salt Tolerance.—Good growth in Czapek solution containing 10 per cent. NaCl. Little or no growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution containing 10 per cent. salt for two weeks the *pH* changes to 5·0.

Gelatine.—Liquefaction in 10 days.

Diastase Production.—Good.

Aspergillus sydowi (Bainer and Sartory) (1, p. 147).

Colonies greenish blue, velvety, with aerial hyphae interlacing and trailing, reverse of colony brick red to orange, becoming dark in old colonies. Stalks usually arise from submerged hyphae, and are up to 500μ × 4–8μ, colourless, smooth, and thick-walled; heads globose, vesicles, 12μ–20μ in diameter, sterigmata in 2 series, conidia globose 3μ–3·5μ in diameter, spinulose. Small heads sometimes present, or penicillate clusters of branched sterigmata on short conidiophores, or single-branched sterigmata sessile on trailing hyphae.

Milk Cultures.—Good growth, acid, coagulation with digestion of curd.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—Fair growth in two weeks in Czapek solution containing 10 per cent. NaCl; little or no growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution containing 10 per cent. NaCl *pH* of medium = 4·0.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Aspergillus terreus Thom (1, p. 150) (2, p. 316) (Pl. 22, figs. 3 and 5).

In addition to the description given in Thom (1), it was noted that in old cultures columnar heads were formed resembling *Aspergillus fumigatus,* but of a smaller size (Pl. 22, fig. 3).

Milk Cultures.—Thick clotting of milk in 3 days. No apparent mycelial growth.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—Good growth in Czapek solution containing 12 per cent. NaCl. Good growth on bottom of flask in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution, with and without the addition of NaCl, the *pH* of the medium varied from 6·2 to 6·7.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Aspergillus glaucus group Link var. (1, p. 101) (Pl. 22, fig. 1, A, B, and C).

Colonies at first yellowish green, later green, velvety, with typical glaucus group head, sterigmata in one series, conidia rough, 7μ × 5μ. Sterigmata sometimes branched, as in fig. 1, B, to form secondary heads.

Heat Tolerance.—No growth when heated to 75° C.

Salt Tolerance.—Growth in Czapek solution containing 5 per cent. NaCl. No growth in Czapek solution containing 10 per cent. NaCl.

pH.—Slight or no change in reaction when grown in Czapek solution for two weeks.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Penicillium brevi-compactum Dierckx var. (12, p. 295) (Pl. 22, fig. 6).

Colonies on nutrient lactose agar are green and zonate. When grown on Czapek agar they agree with Thom's description (12, p. 295):—"Grey-green to fairly deep green, velvety, loose, long stalked, on a thin basal aerial felt up to 1 mm. in depth in centre, margin of conidial area velvety with narrow white margin."

The heads show the characteristically short compact base with divergent sterigmata and conidial chains (see Pl. 22, fig. 6). Conidiophores 4–6μ in diameter, very slightly pitted penicillus, with short primary branch, crowded, and more or less divergent metulae, and sterigmata with loosely parallel, then

tangled chains of conidia 3–5μ in diameter. Our cultures showed spores slightly over the 5μ in diameter. Similar colonies on Richard's agar.

Milk Cultures.—Total digestion, alkaline reaction.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—Slight growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution with and without the addition of NaCl $pH = 6{\cdot}0$.

Gelatine.—Liquefaction in 10 days.

Diastase Production.—Feeble.

Penicillium Johannioli Zaleski (12, p. 391).

This and the following species (*Penicillium cyclopium*) belong to the blue-green subdivision of the Fasciculata group (Thom, 12, p. 391). In the *Penicillium Johannioli* series the zones are narrow, and crowded at the margin, yellow on the reverse, but the colour may be reduced, or even become purplish or black with age.

Colonies on nutrient lactose agar are blue-green, changing when old to smoky colour, at first azonate, then zonate, underside pale to deep yellow in old lactose agar colonies, showing crowded zones, not wrinkled in our cultures—but, according to Thom, may have a few radiate but delicate wrinkles, with the mycelium giving a typically radiate appearance. Conidiophores 400–500μ × 3–4μ, straight, simple, or occasionally branched. Penicillus mostly 40μ–55μ long, with 2 or 3 branches 18μ–32μ × 3μ–4μ, the walls may be smooth or slightly roughened, metulae 10–13μ × 2·5μ–3μ in groups of 4–6, commonly vesicle-like at apex, uneven in length.

Sterigmata about 9μ–10μ × 2μ–2·2μ to 4μ × 2·2μ–3·5μ, mostly subglobose, some ovate, smooth, not persistently adherent in chains or masses.

Milk Cultures.—Coagulation followed by digestion.

Heat Tolerance.—No growth after being heated to 70° C. This mould grew when kept at 5° C. for three weeks.

Salt Tolerance.—Very good surface growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution the *pH* of the medium altered to 5·0. When grown for two weeks in Czapek solution containing 10 per cent. and 15 per cent. NaCl, respectively, the *pH* was 4·0.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Penicillium cyclopium Westling var. (12, p. 384).

Differs from the *Penicillium Johannioli* series in having the zonation of the colony appearing late, and remaining usually indistinct. On Czapek agar the

zonation appears late, the colonies are velvety, thin at the margin, and the centres are sometimes overgrown with white mycelium; the marginal area changes from white during the growing period to a bluish-grey green. The reverse varies from pale yellow when young to purplish shade in centre when old (Czapek agar), or purplish orange (nutrient lactose agar); outer edges in Czapek agar show slight greenish tinge. There is a definite characteristic odour. Conidiophores with rough walls, 3μ in diameter. Penicillus long, about 150μ (Thom = 45–120μ), has a long main stalk, and long appressed branch, or branches, carrying metulae in verticels, sterigmata on end of metulae, conidia are smooth, green, globose, about 3μ in diameter.

Milk Cultures.—Alkaline digestion.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—Grows in Czapek solution containing 10 per cent. NaCl. Yellowish discolouration on under-surface. No growth in Czapek solution containing 15 per cent. NaCl.

pH.—When grown in Czapek solution with and without the addition of NaCl four weeks $pH = 4{\cdot}0$.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Good.

Penicillium sartoryi var. Thom (12, p. 233).

Colonies at first white, then a greenish yellow. Conidiphores long, with an apical group of diverging branches having vesicular apices, from which spring sterigmata and conidia, the latter in a long chain. Sterigmata may arise singly from the fertile hyphae without the presence of a conidiophore, and are irregular in size. Conidia smooth, 4–5μ × 2μ.

Milk Cultures.—Coagulation.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—Slight growth in Czapek solution containing 10 per cent. salt. No growth in Czapek solution containing 15 per cent. NaCl.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Penicillium spinulosum Thom var. (12, p. 183).

Colonies at first green, later smoky brown, under-surface uncoloured, aerial hyphae floccose. Conidiophores long, 150μ–300μ × 3μ–5μ, and bear a single verticel of sterigmata 9·5μ × 11μ–2·3μ. Penicillus is loose column of chains up to 500μ long, conidia are oval to globose, and with thin walls, at first greenish, later a smoky brown, 3·2μ–3·5μ × 3·6μ–4μ.

Milk Cultures.—Coagulation followed by digestion of curd.

Heat Tolerance.—No growth when heated to 65° C. No growth when held at 5° C.

Salt Tolerance.—No growth in Czapek solution containing 10 per cent. NaCl.

pH.—When grown in Czapek solution *pH* of the medium altered to 6·4.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Good.

Gliocladium corda (12, p. 498) (Pl. 22, fig, 7, A and B).

Colonies on Czapek agar velvety-looking, white to dirty pinkish-yellow, ochraceous when mature. Grows in small spreading tufts. Reverse of colony pale yellow. When cultured in flask turns Czapek solution bright yellow. The conidial fructifications are upright, and resemble a Penicillium in structure and production of conidia, except that the conidia are bound by mucus into balls. The conidia are smooth, hyaline, and elliptical, 5μ–7μ × 3μ, and borne on acuminate sterigmata variable in length, generally 10μ–15μ long, springing from conidiophores of variable length, about 3μ in thickness. Considerable confusion exists in the Gliocladium group. Judging by conidial measurements and colour this species described appears to approach *Gliocladium luteolum* von Hohnel. (12, p. 509.)

Milk Cultures.—Alkaline digestion.

Heat Tolerance.—Growth when heated to 75° C. No growth when heated to 80° C. No growth when kept at 5° C. for three weeks.

Salt Tolerance.—When grown in Czapek solution containing 3 per cent. NaCl at end of 7 days a filmy, fluffy, submerged growth, having a dirty white colour. Also a thin growth on surface, with the older parts yellowish. When grown in Czapek solution containing 5 per cent. NaCl a good growth in tiny patches on surface, and submerged budding in cladosporium fashion. Slight growth in Czapek solution containing 10 per cent. NaCl, and little or no growth in Czapek solution containing 15 per cent. NaCl.

pH.—Little or no change when grown in Czapek solution for two weeks with and without the addition of NaCl.

Gelatine.—One-half liquefaction in 10 days.

Diastase Production.—Good.

Trichoderma lignorum Tode (15, vol. 8, p. 110) (Pl. 22, fig. 8, A, B, and C).

Floccose, lumpy, creeping mycelium, at first white, changing through yellow to green. Older cultures powdered over with white sterile mycelium. Conidia rounded, smooth, green, and measure 2·5μ–3μ in diameter, and borne in heads on ends of short conidiophores (Pl. 22, fig. 8A). Chlamydospores about 10μ in diameter (fig. 8, B and C), were found in enormous numbers in the lactose slopes kept below 8° C. These were observed also in the slope kept at 21° C., but in the latter case they were few in number.

These structures had thick hyaline walls, contained refractive bodies, and were either terminal or intercalary on the hyphae. Horne and Williamson describe similar bodies as "macrospores" in their *Eidamia viridiscens,* which Thom considers to be a *Trichoderma species* (Ref. 12, p. 542; Ref. 13, p. 396).

Milk Cultures.—Partial digestion with production of acid in 5 days.

Heat Tolerance.—Growth when heated to 80° C. No growth when heated to 85° C. When kept under 8° C. good growth of sterile mycelium. When kept under 5° C. no apparent growth.

Salt Tolerance.—Poor growth in Czapek solution containing 12 per cent. NaCl. No growth in Czapek solution containing 15 per cent. NaCl.

pH.—Reaction of medium unaltered when grown in Czapek solution with and without the addition of NaCl.

Gelatine.—Partial liquefaction—one-quarter to one-third in 10 days.

Diastase Production.—Good.

This mould was also found growing on deal planks imported for building purposes.

Graphium penicillioides Corda (14) (Pl. 22, fig. 9, A, B, and C).

Appeared as fluffy cushions of white mycelium, conidia appear on branched or unbranched conidiophores (fig. 9c); sometimes they may be produced from mycelium without the presence of conidiophore. Conidia are longish, and variable in size. Some thickened hyphae appear in the colony, and become dark in colour—these also bear conidia, and later from them the graphium type of fructification arises. The length of time of production varies considerably; they are slower on lactose agar than on Czapek or Richard's agar (fig. 9, c).

The graphia (Pl. 22, fig. 9, A and B) are short, stout black cylindrical pillars, bearing a spherical milky white head. Each one arises from swollen cells in mycelium, a thick strand is formed, and the hyphae forming it may be twisted, from the tip of which branch off small colourless conidia. The liquid in which the spores are held is mixable with water. Perithecia did not appear in our cultures.

Milk Cultures.—Strong growth, digestion of curd, acid reaction.

Heat Tolerance.—Slight growth of the cladosporium type when kept for three weeks at 5° C. Better growth when kept at 8° C. for three weeks.

Salt Tolerance.—No growth in Czapek solution containing 20 per cent. NaCl.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Fair.

Coniosporium arundinis Corda (15, vol. 8, p. 558) (Pl. 22, fig. 10, A and B; Pl. 23, fig. 2).

A difficult form to recognise, as growth in media is entirely different from form found on trees; the characteristic spores, with hyaline rim, however, identify it as Coniosporium species. Creeping septate, white mycelium, with

olive green spores, about 6μ long (see Pl. 22, fig. 10B). Spores, which are circular, or subangular in outline and lenticulate, are borne at the tops of conidiophores, each attached by a small outgrowth, which becomes gelatinous and disappears, so that spores have appearance of being borne in heads or clumps. Later all mycelium disappears, and black spots are seen under the medium later: these may lose their rounded appearance and coalesce, forming long streaks (Pl. 23, fig. 2); later they reach the upper surface of the medium. They are then seen to be masses of olive green spores, up to 6·6μ long.

Milk Cultures.—Acid, coagulation followed by digestion.

Heat Tolerance.—No growth when heated to 70° C. Slight growth when kept at 5° C. Fair growth when kept at 8° C.

Salt Tolerance.—Fair growth in Czapek solution containing 12 per cent. NaCl. No growth in Czapek solution containing 15 per cent. NaCl.

pH.—Reaction unaltered when grown in Czapek solution with and without the addition of NaCl.

Gelatine.—Partial liquefaction in 10 days, brownish transparent "acervuli" on surface of medium and sides of tube.

Diastase Production.—Good.

Fusarium species.—Strain A, from butter; Strain B, from butter, microconidial stage; Strain C, from milk.

Fusarium.—Strain A (Pl. 22, fig. 11, A, D, E, and F).

Obtained from sample of butter. Spores sickle-shaped, sharply pointed at each end up to 3 septa (22–35μ × 3·5–4·5μ); sometimes with continuous microconidia. Dr. Ashby (Imp. Bur. Myc.) reports that this form produces on potato mush agar and Quaker oats agar, a carmine plectenchyme, dark blue sclerotia, and creamy sporodochia; while on rice it gives a growth carmine above and brownish yellow below, with sclerotia; chlamydospores are absent or scanty. It appears to be intermediate between sections *Discolor* Wr. and *Lateritium* Wr., the conidia being like those of *F. salicis* Fuck, but that species has not been recorded with carmine plectenchyme.

Milk Cultures.—Acid, coagulation followed by digestion.

Heat Tolerance.—No growth when heated to 70° C. This mould grew when kept at 5° C.

Salt Tolerance.—Growth in Czapek solution with the addition of 12 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—Little or no change when grown in Czapek solution with and without the addition of salt.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

This species was isolated from a sample of butter which had developed reddish patches on surface.

Fusarium.—Strain B (Pl. 22, fig. 11c).

Microconidial stage, which always produced single microconidia $6\mu \times 3\mu$ on all media. Very few macroconidia; these were three septate, and very pointed at the ends. On gelatine, under-surface of colony showed pink colour, on Czapek agar a more orange pink.

Milk Cultures.—Litmus milk decolourised.

Heat Tolerance.—Growth after being heated to 65° C. Slight growth when heated to 70° C. No growth when heated to 75° C. Very slight growth when held at 5° C.

Salt Tolerance.—In Czapek solution containing 3 per cent. NaCl mycelium breaks into rounded spores, fair growth in Czapek solution containing 12 per cent. NaCl, very slight in 15 per cent. NaCl. No growth in 20 per cent. NaCl.

pH.—Little or no change when grown in Czapek solution with and without the addition of NaCl.

Gelatine.—Three-quarters liquefaction in 10 days.

Diastase Production.—Feeble.

A culture of this species was sent to Mr. S. F. Ashby, of the Imperial Bureau of Mycology, who stated that Dr. H. W. Wollenweber had determined it as *F. reticulatum* Mont. (Ann. Sci. Nat., 2nd Ser., **20**, 379, 1843; Wollenweber's Fusaria autographiae delineata, 119–121 and 938, 939). This species is also recorded in Sacc. syll. × 726 as *F. ampelodesmi* Faut et Roum. Other records are on *Beta vulgaris* in Germany, and *Cucumis melo* in Denmark.

Fusarium.—Strain C. (Pl. 22, fig. 11b).

In addition to these, a Fusarium was obtained from milk; this was not reported in our previous paper. The macrospores were more sausage-shaped than those of the Fusarium species found in butter, 5 septate, and not pointed at the ends; spores were larger than the butter form, being $30\mu \times 5\mu$–$6{\cdot}6\mu$ in length.

Milk Cultures.—Very high white fluffy growth with production of acid, coagulation followed by digestion of curd.

Heat Tolerance.—No growth when heated to 70° C. No apparent growth of colony when kept for three weeks at 5° C.

Salt Tolerance.—Fair growth in Czapek solution containing 12 per cent. NaCl; very slight growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—Little or no change when grown in Czapek solution with and without the addition of salt.

Gelatine.—$\frac{1}{8}$ liquefaction in 10 days.

Diastase Production.—Feeble.

Alternaria spp. (Pl. 23, fig. 3).

The stem and spores are rough, and a brown colour, with colourless beaks between the irregularly sized spores, which are in chains. The form belongs to the *A. tenuis* group as defined by Mason, although the spores are sometimes oval rather than obclavate.

Milk Cultures.—Acid, coagulation followed by slow digestion.

Heat Tolerance.—Growth at 70° C. No growth at 75° C.

Salt Tolerance.—Very slight growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown in Czapek solution *pH* of the medium altered to 5·5.

Gelatine.—Slow liquefaction in 12 days.

Diastase Production.—Feeble.

A species of Alternaria was found growing on deal planks imported for building purposes.

Stemphyllium spp. (15, vol. 9, p. 215) (Pl. 22, fig. 12, A and B).

This form resembles *S. ericoctonum* A. Br. and de Bary, but the spores are longer. The hyphae are very jointed, and spores (22–38μ × 8–13μ) smooth, and brownish green in colour.

Milk Cultures.—Alkaline reaction and digestion of curd.

Heat Tolerance.—No growth when heated to 70° C. When kept at 8° C. there was good growth of sterile mycelium. No growth when kept at 5° C.

Salt Tolerance.—Growth in Czapek solution containing 10 per cent. NaCl, slight growth in Czapek solution containing 15 per cent. NaCl. and no growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown for two weeks in Czapek solution $pH = 6{\cdot}6$.

Gelatine.—One-third liquefaction in 10 days.

Diastase Production.—Negative or slightly feeble.

Stemphyllium ericoctonum var. (Pl. 23, fig. 4).

A variety of this species was also found in which the spores are borne in short chains, and are darker and slightly smaller in size, being 21–36μ × 7–9μ. It is thought to be a strain of *S. ericoctonum.*

Milk Cultures.—Alkaline reaction and digestion of curd.

Heat Tolerance.—Growth when heated to 65° C. No growth when heated to 70° C. No growth when kept at 5° C. for three weeks.

Salt Tolerance.—Slight growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—When grown for two weeks in Czapek solution $pH = 6{\cdot}6$.

Gelatine.—One-third liquefaction in 10 days.

Diastase Production.—Negative.

Torula Pers. Sp. (Pl. 22, fig. 13).

A Torula species was found on one occasion with spores 4 × 3·3μ.

Salt Tolerance.—Growth in Czapek solutions containing 5 per cent. and 10 per cent. NaCl. No growth in Czapek solution containing 15 per cent. NaCl.

Diastase Production.—Feeble.

Phoma spp.

Two Phoma spp. were found frequently, but no work was done with them, as they are being investigated by another member of the Botanical Department.

Oidium (*Oospora*) *lactis* Fresen (2, p. 311).

This form was found frequently; generally growing in close contact with yeasts, and difficult to separate into pure culture.

Milk Cultures.—Acid, coagulation and digestion.

Heat Tolerance.—No growth when heated to 60° C.

Salt Tolerance.—Growth in Czapek solution containing 5 per cent. NaCl. Slight growth in Czapek solution containing 10 per cent. NaCl. No growth in Czapek solution containing 15 per cent. NaCl.

Gelatine.—No liquefaction in 10 days.

Diastase Production.—Negative.

Acrostalagmus cinnabarinus Corda (2, p. 312).

Milk Cultures—Alkaline reaction and digestion.

Heat Tolerance.—Growth when heated to 70° C., as a white submerged film. No growth when heated to 75° C. No apparent growth when kept three weeks at 5° C.

Salt Tolerance.—No growth in Czapek solution containing 15 per cent. NaCl.

pH.—Slight or no alteration of reaction when grown in Czapek solution with and without the addition of NaCl.

Gelatine.—Liquefaction in 10 days, brick-red colouration in medium.

Diastase Production.—Good.

Botrytis cinerea Pers. (2, p. 315).

Milk Cultures.—Acid, coagulation, followed by digestion.

Heat Tolerance.—Growth when heated to 65° C. No growth when heated to 70° C. When kept at 5° C. growth normal, but with fluffy sterile mycelium.

Salt Tolerance.—Little or no growth in two weeks in Czapek solution containing 10 per cent. NaCl.

pH.—When grown in Czapek solution the *pH* of the medium altered to 6.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Fair.

Cladosporium spp. (Syn. *Hormodendrum*) (2, p. 313).

We consider with Brooks (4) that the distinction between *Cladosporium* and *Hormodendrum* is a question of the amount and character of the budding of the first formed conidia, and that the two forms gradually merge into one another. Owing to the difficulty of identification, since the type of growth varies with the medium used for cultivation, we were not able to identify the different species met with in the course of this work, but they divided into two chief types—(*a*) a *Cladosporium* type, with larger cells and conidiophores not so complicated as the (*b*) *Hormodendrum* type, with a higher degree of branching in conidiophores and smaller cells.

Milk Cultures.—Thick characteristic growth, partial digestion with production of acid.

Heat Tolerance.—No growth when heated to 60° C.

Salt Tolerance.—Fair growth in Czapek solution containing 10 per cent. and 12 per cent. NaCl. Slight growth in Czapek solution containing 15 per cent. NaCl. No growth in Czapek solution containing 20 per cent. NaCl.

pH.—Little or no change in *pH* when grown in Czapek solution with or without the addition of NaCl.

Gelatine.—Gelatine 50 per cent. liquefied in 10 days.

Diastase Production.—Fair.

Mucor racemosus: Fres. (2, p. 314).

Milk Cultures.—Milk curdled in 48 hours, with decolourisation of the litmus, except on surface, followed by slow digestion of the curd.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C.

Salt Tolerance.—No growth in Czapek solution containing 15 per cent. NaCl.

pH.—When grown in Czapek solution the *pH* of the medium became 6·8.

Gelatine.—Liquefaction in 10 days, green colouration on under-surface.

Diastase Production.—Feeble.

Mucor corymbifer (2, p. 314).

Milk Cultures.—Coagulation with production of acid and soft curd, and separation of whey.

Heat Tolerance.—Growth when heated to 70° C. No growth when heated to 75° C. This mould grew well when kept at 5° C.

Salt Tolerance.—Growth in two weeks in Czapek solution containing 10 per cent. NaCl. Chlamydospores formed in Czapek solution containing 3 per cent. and 5 per cent. NaCl.

pH.—Little or no change in reaction of Czapek solution with and without addition of NaCl.

Gelatine.—Partial liquefaction in 7 days. The mycelium growing in the liquid portion showed an oidium condition, with a green colouration on the under-surface.

Diastase Production.—Feeble.

Stysanus microsporus Sacc. (2, p. 314).

Milk Cultures.—Good growth, but no production of conidial heads, acid reaction.

Heat Tolerance.—No growth when heated to 70° C. No growth when kept at 5° C. for three weeks.

Salt Tolerance.—Growth in Czapek solution containing 10 per cent. NaCl. Little or no growth in Czapek solution containing 15 per cent. NaCl.

pH.—When grown in Czapek solution the *pH* of the medium altered to 6·6.

Gelatine.—Partial liquefaction in 10 days.

Diastase Production.—Feeble.

Sporotrichum carnis (4 and 2, p. 312).

Milk Cultures.—Thin papery growth, slow coagulation followed by digestion.

Heat Tolerance.—No growth after being heated to 60° C. When kept three weeks at 5° C. fairly good growth, pure white colour.

Salt Tolerance.—Good growth in two weeks in Czapek solution containing 5 per cent. NaCl, slight in Czapek solution containing 10 per cent. NaCl, and no growth in Czapek solution containing 15 per cent. NaCl.

pH.—Little or no change in reaction of Czapek solution with or without the addition of NaCl.

Gelatine.—Gelatine 50 per cent. liquefied in 10 days.

Diastase Production.—Feeble.

Trichothecium roseum Link (2, p. 314).

Milk Cultures.—Good growth, production of acid, and finally digestion of the milk.

Heat Tolerance.—Growth when heated to 65° C., no growth after being heated to 70° C.

Salt Tolerance.—Good growth in Czapek solution containing 5 per cent. NaCl; slight growth in Czapek solution containing 10 per cent. NaCl and 12 per cent. NaCl. Little or no growth in Czapek solution containing 15 per cent. NaCl.

pH.—*pH* unaltered when grown for two weeks in Czapek solution with and without the addition of NaCl.

Gelatine.—Gelatine 50 per cent. liquefied in 10 days.

Diastase Production.—Fair.

Chaetomium bostrychoides Zopf (16) (Pl. 22, fig. 14).

This form, which is an extremely variable one, was found on one occasion, perithecia appearing in the cultures. The perithecia are steel grey, oval-shaped, but may vary through all shapes to globose, with black, straight or recurved cirrhi. The lateral hairs are not numerous; encrusted clearly and evenly septate and tapering; olive-brown at the base, nearly 4μ in diameter, and becoming lighter in colour towards the tips, frequently collapsed. The terminal hairs are encrusted and roughened, dark brown to black, and about 4μ in diameter, and generally spirally coiled, irregularly septate, and with a few septate and spirally coiled branches.

When young the spores are hyaline, greenish, with granular contents; when mature, oval or almost spherical, sometimes apiculate, or rounded at both ends, 4 $\times$ 5μ in our type.

Milk Cultures.—Alkaline digestion.

Heat Tolerance.—Growth when heated to 60° C. No growth when heated to 70° C.

Salt Tolerance.—Slight growth in Czapek solution containing 5 per cent. NaCl. No growth when 10 per cent. NaCl is present.

pH.—When grown in Czapek solution containing 5 per cent. NaCl there was little or no change.

Gelatine.—Partial liquefaction.

Diastase Production.—Feeble.

DISCUSSION.

It is a well-known fact that salted butter is less liable to become mouldy than unsalted; and unsalted butter made from sweet cream may be regarded as a suitable medium for mould growth under favourable conditions.

While it is recognised that the heat tolerance and salt tolerance figures only hold good for the conditions under which they were carried out, the results obtained show the range that exists among the various species of fungi. *Oidium lactis* is killed when heated to 60° C., while some fungi are not killed at 75° C. Most fungi do not grow at 5° C., but a species of Penicillium, Fusarium, *Mucor corymbifer,* and *Sporotrichum carnis* grew when held at this temperature. Practically all the fungi grew in Czapek solution that contained 10 per cent. NaCl, most of them were inhibited by the addition of 15 per cent. NaCl, and all were inhibited by the addition of 20 per cent. NaCl. The inference from this is that 2·3 per cent. NaCl, or over, is necessary in butter to inhibit the growth of fungi.

Since moulds are aerobic, they usually show their presence by discoloured patches on the surface, and reduce the market value of the butter. The present investigation shows the large number of species that may occur in butter and that can survive under unfavourable conditions, since all the fungi described

were found in butter taken from the interior of a 56 lb. box two weeks old, and that had, in many cases, been put in cold storage, and in no case held at a temperature exceeding 15° C. Although many of these fungi had previously been recorded as found in milk (2), it is regarded as unlikely that the butter was contaminated from this source, as the cream is pasteurised and cooled before churning, although it could occur with inefficient pasteurisation.

The pasteurisation temperature ranges from 85° C.–95° C., and no mould was found to survive this temperature. The probable explanation is that these types of fungi are widespread, and get into the cream either after pasteurisation, or during churning, or into the butter after manufacture. The water used for washing the butter may be a source of contamination, since it is sometimes stored in an uncovered tank. The churn is considered to be the chief piece of equipment responsible for mould trouble in the creamery. Crevices and cracks gradually develop in the wood and rollers, in which fungi can thrive, and only those who have tried can realise the difficulty of sterilising a churn.

Air contamination is another cause. Creameries are generally situated by the road-side, which increases the risk of contamination by air-borne fungal spores. In the creamery the machinery in motion keeps the air in circulation, and spores of fungi growing in damp or neglected places are blown about, and thus get into the cream or butter. The cream, after pasteurisation, is exposed to contamination from the air, since it flows over a water- or brine-cooler, and is further usually cooled in an open vat. The butter is exposed while being transferred from the churn to the boxes, and the packed boxes are left for a varying period of time standing on the floor before the lid is put on.

Owing to the provisions of the Dairy Produce Act, 1924, wood, parchment, and salt are considered to be negligible factors, unless they have been left exposed in a damp place.

Attention is drawn to the fact that when moulds were sent for confirmation as to species, those to whom they were sent remarked in many cases that, while they closely resembled a certain species, they did not conform altogether to the type species.

This is believed to show that the Irish fungi, as a result of insularity, have developed characteristics of their own.

Summary.

In the course of an investigation into the causes of deterioration of butter made from sweet cream, a study was made of the fungi found. The following were identified :—

(1) *Aspergillus fumigatus* Fres.
(2) *Aspergillus luteo-niger* Lutz
(3) *Aspergillus sydowi* Bainer and Sartory
(4) *Aspergillus terreus* Thom
(5) *Aspergillus glaucus* group Thom
(6) *Penicillium brevi-compactum* Dierckx var.

(7) *Penicillium johannioli* Zaleski
(8) *Penicillium cyclopium* Westling var.
(9) *Penicillium sartoryi* Thom
(10) *Penicillium spinulosum* Thom var.
(11) *Gliocladium* spp. Corda
(12) *Trichoderma lignorum* Tode
(13) *Graphium penicillioides* Corda
(14) *Coniosporium arundinis* Corda
(15) *Fusarium* spp. including *F. reticulatum* Mont.
(16) *Alternaria* spp.
(17) *Stemphyllium* spp. Wallr. including *S. ericoctonum.*
(18) *Torula* spp. Pers.
(19) *Phoma* spp.
(20) *Oidium lactis*
(21) *Acrostalagmus cinnabarinus* Corda
(22) *Botrytis cinerea* Pers.
(23) *Cladosporium* spp.
(24) *Mucor racemosus* Fres.
(25) *Mucor corymbifer* Cohn
(26) *Stysanus microsporus* Sacc.
(27) *Sporotrichum carnis* Brooks and Hansford.
(28) *Trichothecium roseum* Link
(29) *Chaetomium bostrychoides* Zopf.

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Plate 22.

Fig. 1a. Irregular head of *Aspergillus glaucus* var. showing branches from which secondary heads will be produced.
Fig. 1b. Later stage of similar irregular head showing appearance of secondary heads.
Fig. 1c. Warted spores of above.
Fig. 2. *A. fumigatus* head drawn to scale for comparison with *A. terreus*, fig. 3.
Fig. 3. Columnar head of *A. terreus* drawn to same scale as *A. fumigatus*, fig. 2.
Fig. 4. Diagram of arrangement of *A. fumigatus* showing flask-shaped vesicle and single row of sterigmata.
Fig. 5. Diagram of arrangement of *A. terreus* showing double row of sterigmata and spherical vesicle.
Fig. 6. Head of *Penicillium brevi-compactum* showing characteristic asymmetrical appearance.
Fig. 7a. Penicillus-like head of *Gliocladium* sp.
Fig. 7b. Appearance of mucus-covered heads under high power.
Fig. 8a. High power appearance of *Trichoderma lignorum.*
Fig. 8b. Intercalary chlamydospore grown at low temperature.
Fig. 8c. Terminal chlamydospore of *Trichoderma lignorum* grown at low temperature.
Fig. 9a & 9b. *Graphium penicillioides* Corda, fructification general appearance.
Fig. 9c. Cladosporium-stage of *Graphium penicillioides.*

Fig. 10A. *Coniosporium arundinis* Corda spores.
Fig. 10B. Appearance of young fructification of *Coniosporium arundinis* Corda under high power.
Fig. 11A. *Fusarium* spore strain A.
Fig. 11B. *Fusarium* spore strain C.
Fig. 11C. Microconidial stage of *Fusarium* sp.
Fig. 11D, E, F. Continuous spores of Fusarium strain A grown in Czapek solution.
Fig. 11G. Continuous chain of microspores of *Fusarium* (strain B).
Fig. 12A. Appearance of *Stemphyllium ericoctonum.*
Fig. 12B. Rounded mycelial cells.
Fig. 13. *Torula* sp., appearance under high power.
Fig. 14. *Chaetomium bostrychoides* Zopf. Appearance of perithecium.

PLATE 23.

Fig. 1. Microphotograph of head of *Aspergillus fumigatus.*
Fig. 2. *Coniosporium arundinis* culture—showing streaked appearance due to acervuli.
Fig. 3. Microphotograph of *Alternaria* sp.
Fig. 4. *Stemphyllium ericoctonum* strain with small spores.

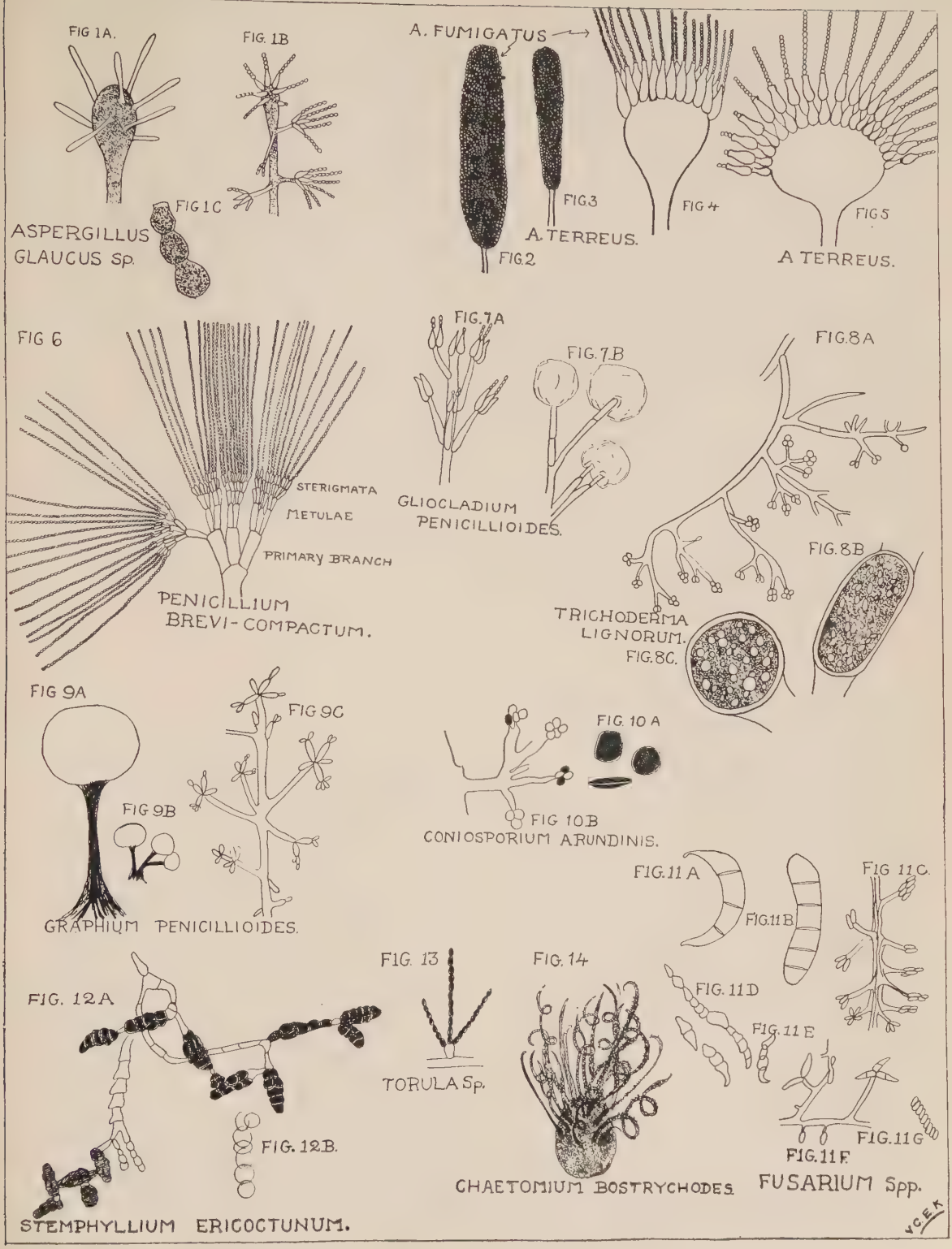
FIG 1A.
FIG. 1B
FIG 1C
ASPERGILLUS GLAUCUS Sp.
A. FUMIGATUS
FIG.2
FIG.3
A. TERREUS.
FIG 4
FIG 5
A TERREUS.
FIG 6
STERIGMATA
METULAE
PRIMARY BRANCH
PENICILLIUM BREVI-COMPACTUM.
FIG.7A
FIG.7B
GLIOCLADIUM PENICILLIOIDES.
FIG.8A
FIG.8B
TRICHODERMA LIGNORUM.
FIG.8C.
FIG 9A
FIG 9B
FIG 9C
GRAPHIUM PENICILLIOIDES.
FIG 10 A
FIG 10B
CONIOSPORIUM ARUNDINIS.
FIG.11 A
FIG.11B
FIG 11C.
FIG. 11D
FIG. 11 E
FIG.11G
FIG.11F
FUSARIUM Spp.
FIG. 12A
FIG. 12B.
STEMPHYLLIUM ERICOCTUNUM.
FIG. 13
TORULA Sp.
FIG. 14
CHAETOMIUM BOSTRYCHODES.

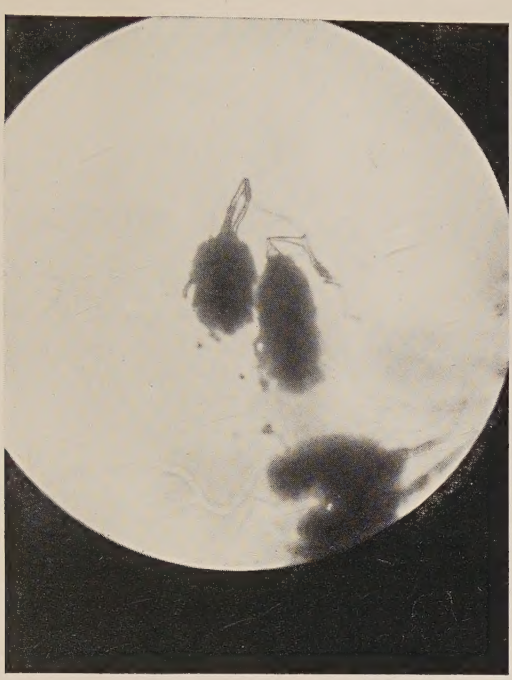

FIG. 1.

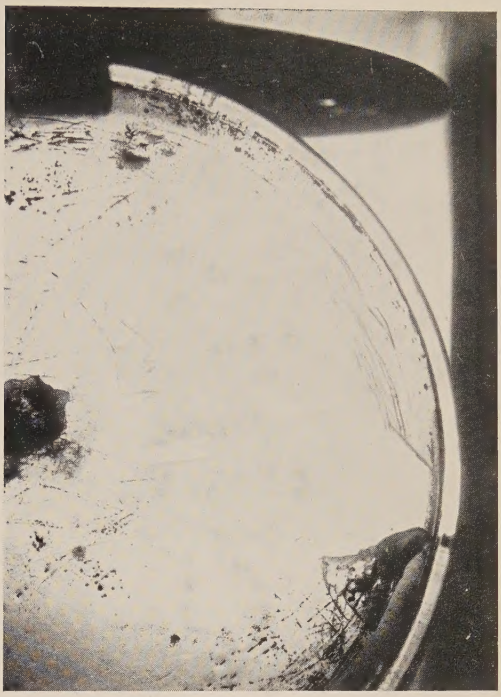

FIG. 2.

FIG. 3.

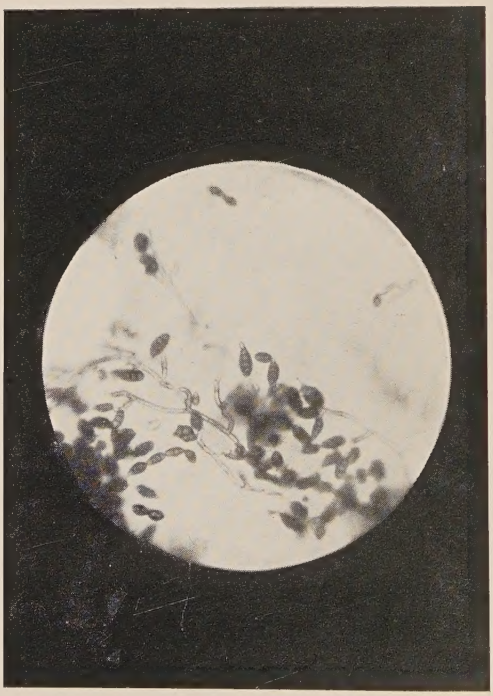

FIG. 4.